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Maternal and Fetal Exposure to Bisphenol A Is Associated with Alterations of Thyroid Function in Pregnant Ewes and Their Newborn Lambs

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The putative thyroid-disrupting properties of bisphenol A (BPA) highlight the need for an evaluation of fetal exposure and its consequence on the mother/newborn thyroid functions in models relevant to human. The goals of this study were to characterize in sheep a relevant model for human pregnancy and thyroid physiology, the internal exposures of the fetuses and their mothers to BPA and its main metabolite BPA-glucuronide (Gluc), and to determine to what extent it might be associated with thyroid disruption. Ewes were treated with BPA [5 mg/(kg · d) sc] or vehicle from d 28 until the end of pregnancy. Unconjugated BPA did not appear to accumulate in pregnant ewes, and its concentration was similar in the newborns and their mothers (0.13 ± 0.02 and 0.18 ± 0.03 nmol/ml in cord and maternal blood, respectively). In amniotic fluid and cord blood, BPA-Gluc concentrations were about 1300-fold higher than those of BPA. Total T_4 concentrations were decreased in BPA-treated pregnant ewes and in the cord and the jugular blood of their newborns (30% decrease). A similar difference was observed for free T_4 plasma concentrations in the jugular blood of the newborns. Our results show in a long-gestation species with a similar regulatory scheme of thyroid function as humans that BPA *in utero* exposure can be associated with hypothyroidism in the newborns. If such an effect were to be confirmed for a more relevant exposure scheme to BPA, this would constitute a major issue for BPA risk assessment. (*Endocrinology* 154: 521–528, 2013)

Bisphenol A (BPA), a major molecule used in the plastic industry, is now acknowledged as an endocrine disruptor that could exert deleterious effects on human health (1, 2). Most investigations have focused on reproductive functions based on the estrogen-mimetic properties of this compound. However, evidence has accumulated that BPA might have negative effects on other endocrine systems (3, 4) including thyroid function (5). Epidemiological data obtained on a cohort of men from infertile couples (6) and on a larger cohort of healthy individuals (National Health and Nutrition Examination Survey cohort) (7) suggests that BPA exposure might be associated with some degree of thyroid dis-

ruption characterized by a suggestive inverse relationship between urinary BPA concentrations and total T_4 concentrations.

In vivo studies in mammals looking at the effect of BPA on thyroid function are scarce, and they have all been performed in rodents. In one study, the physiological rise in circulating total T_4 that occurs around postnatal d 15 (PND15) was amplified in animals born to and fed by mothers treated with BPA from gestational d 6 (GD6) until weaning with an oral dose at 1 mg/(kg · d), *i.e.* below the current no-observed-adverse-effect level of 5 mg/(kg · d) (8). These changes in circulating T_4 were associated with changes in the expression of neurogranin, a thyroid-reg-

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Abbreviations: AUC, Area under the curve; BPA, bisphenol A; CV, coefficient of variation; GD6, gestational d 6; Gluc, glucuronide; LOQ, limit of quantification; PK, pharmacokinetics; PND 15, postnatal d 15; TH, thyroid hormone.

ulated marker for neurodifferentiation, in the dentate gyrus within the hippocampus. In another study in pregnant rats (9), BPA oral treatment at 0.1 mg/liter in drinking water [approximately 10 $\mu\text{g}/(\text{kg} \cdot \text{d})$] caused a transitory decrease in the free T_4 concentration in mothers in the early postpartum period (PND0–PND7), whereas their male offspring showed an increase in free T_4 at PND7 followed by a decrease in free T_4 at PND21. The significance of these studies in terms of the risk analysis for human health is limited for two main reasons. First, the rat is often considered controversial in terms of its relevance to thyroid regulation in humans, in particular for development-related issues, because of a difference in the timing of the ontogenesis of thyroid function. Second, these two studies did not provide information regarding the internal exposure of the animals, impeding all comparisons with known human exposure.

Evidence for the potential of BPA as a thyroid disruptor has also arisen from results obtained with mammalian cells and amphibian or fish models *in vivo*. These studies converge to suggest that BPA can exert an antagonistic effect on thyroid hormone (TH)-dependent mechanisms (10–16). This effect appears to be mediated, at least in cell models, by the increased recruitment of corepressor of the TH nuclear receptors from the N-cor family (17, 18).

Overall, it can be considered that uncertainties remain regarding the potential of BPA as a thyroid disruptor *in vivo* in mammals. Thus, the goal of this study was to test the hypothesis that exposure to BPA *in utero* can lead to thyroid disruption in both mothers and newborns and to characterize at the same time the exposure of different maternal and fetal compartments. To achieve this goal, we used the sheep model as an animal exhibiting similar patterns of thyroid axis ontogenesis and regulation as humans.

Materials and Methods

Chemicals

Chemicals were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France) unless otherwise specified.

BPA stock solutions (500 mg/ml, five different solutions used throughout the experiment) were prepared by dissolving BPA (99% purity) in absolute HPLC-grade ethanol. Solutions were kept at +4°C in sealed amber glass bottles. Solutions for sc administration (91 mg/ml) were prepared weekly by a 5.5-fold dilution of a stock solution in corn oil (Sigma). Solutions were kept at +4°C. Vehicle solutions were obtained by mixing ethanol with corn oil. For iv administration, the stock solution was diluted 10-fold in propandiol to obtain a 50 mg/ml solution.

Animal husbandry and treatments

The study was conducted on adult ($n = 15$, 2–5 yr old) Lacaune ewes in the sheep research facility of the National Vet-

erinary School of Toulouse, France. These ewes had multiple pregnancies before being included in the experiment. Ewes were submitted to hormonal synchronization of their estrous cycle and were artificially inseminated with fresh Lacaune ram sperm. Then, a Blackbelly ram was introduced into the flock to mate with the nonpregnant ewes. Finally, five vehicle- and six BPA-treated ewes got pregnant after the artificial insemination procedure, and two others from each group got pregnant at the following cycle (about 2 wk later) after mating the ram.

Pregnant ewes were randomly allocated to two groups: vehicle or BPA treated. Groups were balanced for body weights and fecundation modality (insemination *vs.* ram). Animals from the BPA group received daily sc injections 5 mg/(kg \cdot d) of BPA starting at GD28 and until the end of pregnancy, whereas the control group received an equivalent volume of vehicle. The administered volume was regularly adjusted for the most recently recorded body weight. The 93rd injection was performed iv to document BPA toxico-kinetic parameters in pregnant animals.

Ewes were fed hay *ad libitum* and vegetable pellets. The quantity of pellets was adjusted monthly to fulfill pregnancy requirements. The animals were kept inside under natural photoperiodic and temperature conditions.

Starting at GD145, lambs were delivered by cesarean section aseptically performed under spinal anesthesia (75–125 mg lidocaine aseptically injected in the sacrolumbar cisterna; Lurocaine, Vetoquinol SA, Lure, France) and immediately presented to their mother. As soon as the umbilical cord had been cut, the ewes received an iv injection of a nonsteroidal antiinflammatory (flunixin meglumine 1 mg/kg iv; Meflosyl Fort Dodge Santé Animale, Tours, France) and an antibiotic (amoxicillin long action 20 mg/kg im; Longamox, Vetoquinol) to prevent pain and septic complication. Cesarean section was performed to ensure a better control of the birth process without hormonal treatment and to allow placenta and cord blood sampling in good conditions.

All animal procedures were carried out in accordance with the accepted standards of humane animal care under the agreement number 31-242 for animal experimentation from the French Ministry of Agriculture.

Monitoring BPA exposure and thyroid status throughout pregnancy

Blood samples were collected twice a week from the beginning until the end of treatments, just before a new injection. Serial blood samples were collected at 0, 2, 4, 6, 8, 20, and 24 h after the first, 30th, and 58th BPA sc injections to document BPA pharmacokinetics (PK). The 93rd injection was performed iv, and serial samples were collected at 0, 2, 4, 8, 15, and 30 min and 1, 2, 4, 6, 8, 10, and 24 h after the administration to determine BPA PK parameters and the bioavailability for the sc route.

To avoid any confusion linked to differences in gestational stages, only the samples collected from the ewes fecundated by artificial insemination were included in the PK analysis and the evaluation of the thyroid status during pregnancy. Total and free T_4 and TSH were assayed in one in every four samples collected twice a week.

BPA exposure of the fetal compartments

During surgery, whenever possible, amniotic fluid, placenta samples from intercotyledon spaces, mixed cord blood from both the umbilical vein and artery, and colostrum were collected

to assay BPA and BPA-glucuronide (Gluc). The frozen placenta samples were homogenized in ultrapure water (1 ml water/g tissue) using a tissue homogenizer (Ultra-Turrax T25, IKA Labortechnik, Staufen, Germany, maximum speed for 1 min in an ice bath). The total extract was kept in an ice bath and treated like serum samples. Recovery of the method for both BPA and BPA-Gluc was evaluated by assaying the molecules in a placenta from a control vehicle-treated ewe homogenized in water spiked with either BPA or BPA-Gluc. The BPA concentration in the extract spiked with BPA at 100 ng/ml was 111 ng/ml, and the BPA-Gluc concentration in the extract spiked with BPA-Gluc at 1000 ng/ml was 1093 ng/ml.

Thyroid status of ewes and lambs at birth

For the ewes, total T_4 and T_3 and free T_4 were assayed in the jugular blood collected at the time of cesarean section.

Three samples (1 ml) of the lamb jugular blood were sequentially collected within the first hour of life to monitor the endocrine status of the newborn. Free and total T_4 and total T_3 were measured in the serum from the cord blood and those three samples. TSH was assayed in cord blood samples only. At 2 months of age, blood was collected every 3 h for 12 h to evaluate the potential recovery of the thyroid function.

BPA and BPA-Gluc assay

BPA was assayed by ultra-performance liquid chromatography tandem mass spectrometry as previously described with a method validated for sheep plasma samples according to U.S. Food and Drug Administration requirements (19). BPA and BPA-Gluc were assayed in one of every four samples collected twice a week in ewes and all the samples collected at birth using a novel assay method (20). The BPA and BPA-Gluc within- and between-day coefficients of variation (CV) measured for three different concentration levels were lower than 13% for both assay methods. BPA limit of quantification (LOQ) for the first method was validated in plasma at 1 ng/ml, and the accuracy of the test ranged from 102–111%. For the second method, the LOQ were validated at 1 and 20 ng/ml for BPA and BPA-Gluc, respectively. The method accuracies in serum ranged from 95–108% and 91–102% for BPA and BPA-Gluc, respectively.

TH and TSH assays

Total and free plasma T_4 and total plasma T_3 concentrations were determined using RIA kits (Coat-A-Count kits; Siemens Healthcare Diagnostics, Los Angeles, CA). The mean intraassay and interassay CV of three quality control pools were less than 10 and 15% for total T_4 , respectively, for all assays. The LOQ for total T_4 and T_3 assays were validated at 5 and 0.1 ng/ml, respectively, as the lowest values determined with a CV lower than 20%. The limit of detection of the free T_4 assay was set at the lowest value of the standard curve, *i.e.* 1 pg/ml.

TSH was assayed in 200- μ l plasma aliquots in duplicate with a heterologous RIA using a double-antibody separation method with reagents provided by the National Hormone and Pituitary Program (Dr. A. Parlow, Harbor UCLA, Torrance, CA) according to the National Hormone and Pituitary Program-recommended procedure. Results are expressed in terms of the NIDDK-bTSH-1-2 standard. The limit of assay sensitivity was

evaluated at 0.2 ng/ml of the NIDDK-bTSH-1-2 standard. The mean intra- and interassay CV for three quality control pools were less than 15%.

PK analyses

The time course of BPA concentrations in samples collected twice a week and serial samples collected after sc injections were fitted to a biexponential equation corresponding to a mono-compartmental model for repeated extravascular administrations with weighting of the data by the inverse square of the estimated values ($1/\hat{y}^2$). The estimated parameters were used to simulate the daily fluctuations of BPA plasma concentrations throughout the treatment.

The time course of BPA concentrations in serial samples collected for 24 h after the iv injections were fitted to a biexponential equation corresponding to a bi-compartmental model with a $1/\hat{y}^2$ weighting of the data to determine elimination half-life, plasma clearance, and distribution volume at steady state.

The 24-h postinjection area under the curve (AUC_{0-24h}) after the 58th sc administration and the 93rd iv administration was used to calculate the BPA bioavailability by the sc route. For both routes, the AUC_{0-24h} estimated by the model were corrected by the AUC that accounted for the residual concentrations measured just before the new administration. Subcutaneous bioavailability was defined as the ratio of the corrected AUC_{0-24h} for the sc route to the equivalent AUC_{0-24h} for the iv route expressed as a percentage. The ratio of the AUC_{0-24h} after the first administration to the one subsequent to the 58th injection was used as an index for BPA accumulation. All PK analyses were done using WinNonlin version 5.3 software (Pharsight Corp., Mountain View, CA).

Statistical analysis

The effect of the treatment on the time course of TH concentrations in pregnant ewes was analyzed by a two-way ANOVA with treatment and sampling time and their interactions as fixed-effect factors and animal nested within treatment as a random-effect factor. The effect of treatment on TH concentrations in samples collected at birth was analyzed using a two-way ANOVA with sex, treatment, and their interactions as fixed-effect factors. The statistical analysis did not account for twin occurrence. All statistical analyses were done using general linear models on SYSTAT12 software (Systat Software Inc., San Jose, CA).

Results

Results are given as mean \pm SE values.

Maternal exposure to BPA throughout pregnancy

According to the model predictions, the daily BPA concentrations were estimated to fluctuate between a maximum mean concentration of 240 ± 15 ng/ml and a minimum of 38 ± 4 ng/ml. The maximum concentrations were reached on average at 94 ± 6 min after the sc administration. The ratio of the AUC_{0-24h} for the 58th sc injection and the first injection was 0.97, indicating that BPA did not accumulate in pregnant ewes.

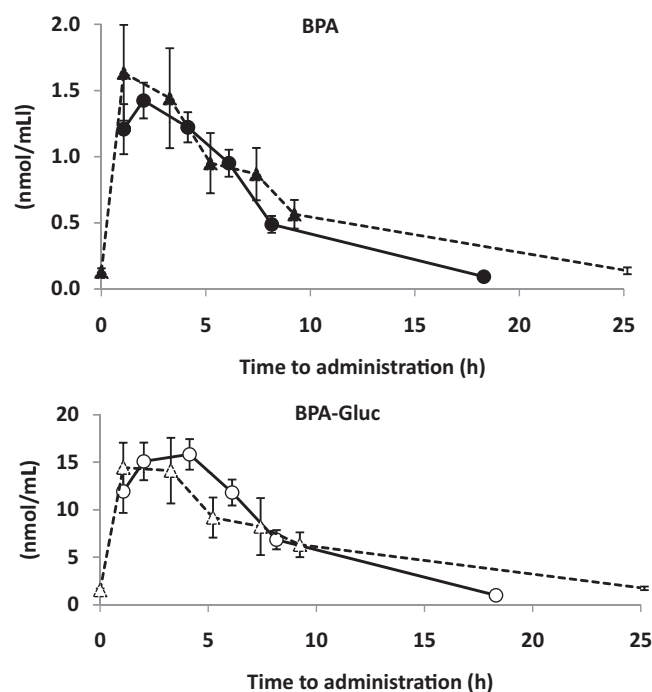


FIG. 1. Time course of mean (\pm SE) serum BPA (closed symbols) and BPA-Gluc (open symbols) concentrations in pregnant ewes ($n = 6$) treated with daily injections of BPA [5 mg/(kg \cdot d) sc] from GD28 until the end of pregnancy. BPA and BPA-Gluc were assayed simultaneously in serial blood samples collected just after the first (solid line) and the 58th (dotted line) administrations.

BPA clearance by iv route was elevated (12.2 ± 1.2 ml/(kg \cdot min)], and its terminal half-life was short (131.2 ± 10.8 min). The distribution volume at steady state (917.5 ± 217.0 ml/kg) was higher than the volume of extracellular fluid. The bioavailability for the sc route was evaluated at $35 \pm 3\%$.

Figure 1 shows the time course of BPA and BPA-Gluc molar serum concentrations assayed in the serial samples collected after the first and the 58th sc administrations. The time course of both BPA and BPA-Gluc concentrations was very similar between the two administrations. Furthermore, the mean ratios of BPA-Gluc to BPA concentrations were very similar between the two administrations (mean ratio 37 and 35 for the first and the 58th administrations, respectively), indicating that the transformation rate of BPA into BPA-Gluc did not differ between these two gestational stages.

BPA materno-fetal exposure at birth

One lamb showed a very high concentration of free BPA in amniotic fluid compared with the other animals (lamb 19, 5.4 nmol/ml). A Grubb's test indicated that this value was an outlier leading to the exclusion of this animal for BPA and BPA-Gluc data from amniotic fluid. The concentrations in BPA and BPA-Gluc in cord blood and placenta samples from this animal were within the range of the values observed for all other animals and were thus kept in the analysis. BPA serum concentrations were very similar between mothers and lambs (Table 1). By contrast, BPA-Gluc concentrations were about 30- and 50-fold higher in cord blood and amniotic fluid, respectively, than in maternal blood at delivery.

Thyroid status of the mother and offspring

Figure 2 shows the time course of total and free T_4 serum concentrations in pregnant ewes throughout the treatment. There was a significant interaction between the time and the treatment on total T_4 concentrations with decreased concentrations in BPA-treated ewes, but there was no such effect on serum free T_4 concentrations.

For all TH and samples assayed at the time of birth, there was no interaction between the sex and the treatment. Total and free T_4 concentrations were higher in the lamb cord and jugular blood at birth than in the mother's blood (Fig. 3). BPA *in utero* exposure resulted in a significant decrease in jugular blood concentrations of 29 and 32% for total and free T_4 , respectively, within the first hour of life (Fig. 3) and a 33% decrease in total T_4 in cord blood. Total T_3 concentration in samples from the first hour of life showed a trend ($P = 0.063$) toward lower values in BPA-treated lambs. At 2 months of age, the TH blood concentrations did not differ between groups ($P > 0.05$).

TSH concentrations were very rarely above assay sensitivity in both maternal and newborn samples. Too few values were available to perform analysis. The only indication from this result is that BPA pregnancy exposure was not associated with a dramatic increase in TSH secretion, although we cannot rule out that a moderate nondetectable increase might have occurred.

TABLE 1. BPA and BPA-Gluc concentrations in maternal and fetal samples collected at delivery from ewes treated with BPA [5 mg/(kg \cdot d) sc from GD28 until the end of gestation]

	Maternal blood (nmol/ml), $n = 6$	Cord blood (nmol/ml), $n = 11$	Amniotic fluid (nmol/ml), $n = 7$	Placenta (nmol/g tissue), $n = 10$	Colostrum (nmol/ml), $n = 6$
BPA	0.18 ± 0.03	0.13 ± 0.02	0.24 ± 0.04	6.54 ± 1.71	0.21 ± 0.03
BPA-Gluc	5.73 ± 1.45	161.72 ± 9.13	312.11 ± 85.86	67.09 ± 10.52	1.69 ± 0.53
BPA-Gluc/BPA	36 ± 12	1380 ± 184	1331 ± 283	18 ± 6	7 ± 2

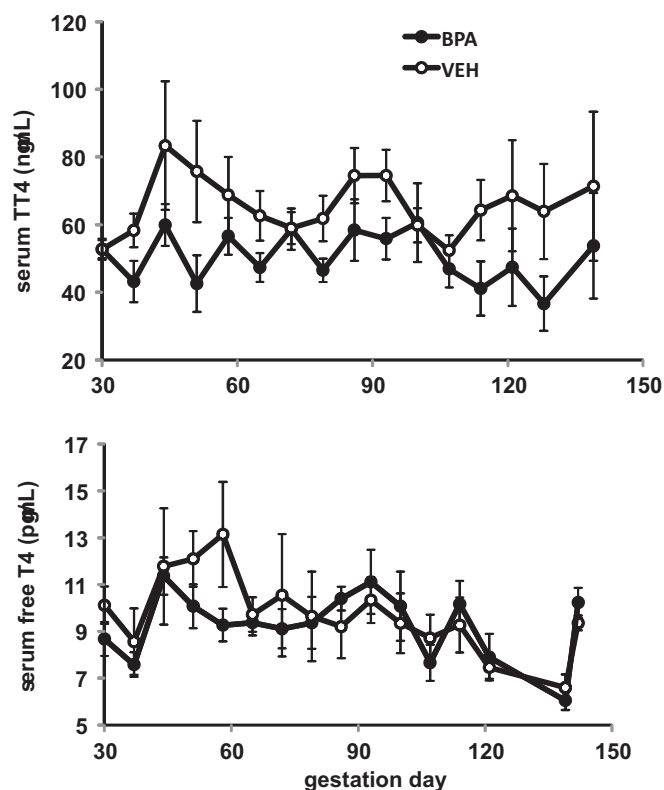


FIG. 2. Time course of mean (\pm SE) total (top) and free (bottom) T₄ serum concentrations in pregnant ewes treated with vehicle (VEH) (○; n = 5) or BPA [●; 5 mg/(kg · d) sc from GD28 until the end of pregnancy; n = 6]. T₄ was assayed in blood samples collected weekly throughout the treatment period. The time course of mean total T₄ (TT4) concentrations differed significantly between the two groups (interaction time \times treatment $P < 0.05$).

Discussion

Experimental data indicate that BPA can cross the placental barrier (21, 22). Furthermore, in the rat, BPA-Gluc also appears to cross this barrier in small amounts, whereas the fetus can exhibit some deconjugation activity toward BPA-Gluc (23) concomitantly with very low conjugation activity during early development. Altogether those results led to the hypothesis that the fetus, more particularly during the earliest stages of development, could be at risk for overexposure to bioactive BPA. Our results indicate that although BPA is transported from the mother to the fetus through the placenta, the fetus is not overexposed to unconjugated BPA consequent to the maternal treatment. This observation is fully consistent with biomonitoring data obtained in mother/newborn human cohorts indicating that the newborns exhibit BPA blood concentrations very similar that of their mothers (24, 25). This rules out the hypothesis of overexposure of the fetus to unconjugated BPA for the late gestation stages but cannot, however, completely rule out the possibility of fetal overexposure for earlier stages when hepatic conjugation activity

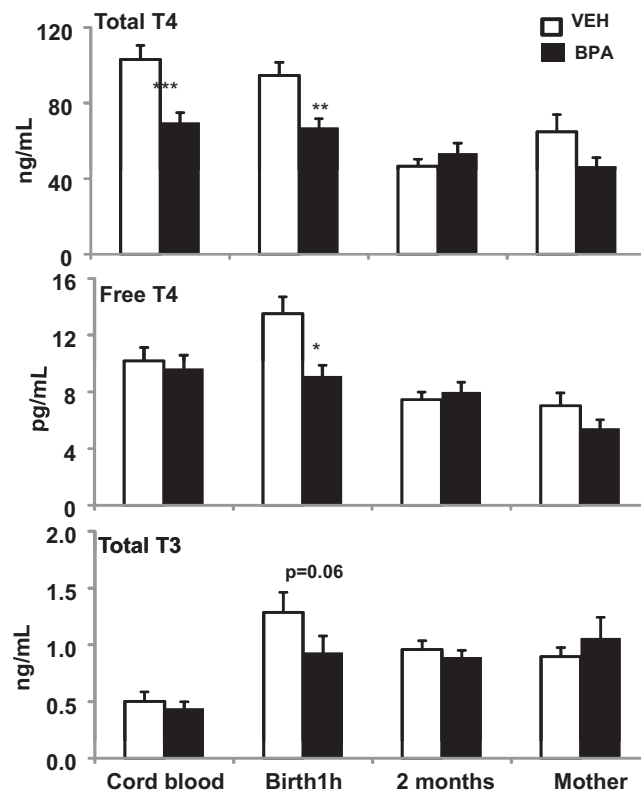


FIG. 3. Mean (\pm SE) serum total T₄, free T₄, and total T₃ concentrations in newborn lamb cord blood and jugular blood within the first hour after birth (Birth 1 h) and at 2 months of age and in maternal blood at delivery. Lambs were born to vehicle (VEH)-treated (white bars) or BPA-treated (black bars) mothers [5 mg/(kg · d) sc from GD28 until the end of pregnancy]. Treatment effect: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n = 9 and 12 for cord blood, 15 and 11 for the 1-h birth samples, and 11 and 11 for serial samples at 2 months of age for vehicle and BPA lambs, respectively, and 8 and 6 for vehicle and BPA mothers, respectively.

is still very limited. Neither unconjugated BPA nor BPA-Gluc accumulates during the course of pregnancy in mothers. The BPA half-life obtained in our study was very similar to the one previously evaluated in prepubertal female lambs (19). The large volume of distribution of unconjugated BPA reported in our study suggests that unconjugated BPA is widely distributed within the organism of the pregnant ewe.

In the present study, unconjugated BPA concentrations were much higher in the placenta than in maternal blood. In the rat, both the placenta and the fetus express deconjugation activity (23). Thus, our result could be explained by the existence of deconjugation activity within the ewe placenta. If this were to hold true, it would mean that the placenta itself might be the primary source of unconjugated BPA for the fetus. In our study, BPA concentrations in the newborn were within the same range as those of their mothers (Table 1), suggesting that, at least toward the end of gestation, the fetus can overcome partially the continuous supply of

unconjugated BPA by triggering detoxification mechanisms such as phase II metabolism reactions. Such a mechanism could explain the particularly high levels of BPA-Gluc measured in all fetal compartments.

In contrast to BPA, the newborns were indeed much more exposed to the glucuronide conjugate than their mothers. The very high levels of BPA-Gluc encountered in both the newborn blood and amniotic fluid suggest that BPA-Gluc is trapped once in the fetal compartment. The source of BPA-Gluc in the fetus remains to be elucidated. On the one hand, BPA-Gluc might be from maternal origin. On the other hand, this BPA-Gluc might be of fetal origin. UDP-glucuronosyl-transferase activity in the liver starts to develop during fetal life and continuously increases until birth (26) and so could have contributed to BPA-Gluc production in the fetal compartments in particular toward the end of pregnancy. In addition, the amniotic fluid might constitute a nonnegligible source of BPA-Gluc to the fetus after oral ingestion. Indeed, the fetus is ingesting about 900 ml of amniotic fluid per day (27), and it can be estimated in our study that this might provide as much as 100 mg of BPA-Gluc per day at the end of the treatment period. Given the very high accumulation of BPA-Gluc in the fetal compartment, the question remains of the potential deconjugation of BPA-Gluc directly by the target tissues of the fetus. Such a process could indeed lead to an *in situ* reactivation of the conjugate, resulting in an overexposure of sensitive target tissues to free bioactive BPA.

Our results showed that maternal and fetal exposure to BPA is associated with disruption of the thyroid function of both the mothers during pregnancy and the newborns characterized by a decrease in circulating total and/or free TH levels. Those results are consistent with epidemiological data in humans showing a suggestive inverse relationship between urinary BPA concentrations and total T_4 in adults (7) and one study in rodents showing a transitory decrease in free T_4 concentrations in early postpartum stages in dams treated with low doses of BPA during pregnancy (9). Another *in vivo* study showed an amplification of the physiological rise in total T_4 occurring around PND15 in young rats born to and fed by mothers treated with BPA (8). It is very likely that the apparent discrepancy between this result and our results in the sheep lies, at least in part, in interspecies differences in thyroid function ontogenesis and regulation.

Free T_4 concentrations are controlled solely by clearance mechanisms and T_4 entrance rate in the blood, *i.e.* secretion by the thyroid gland (28), whereas bound and therefore total T_4 concentrations can also be modulated by displacement mechanisms from the specific binding proteins, T_4 -binding globulin and/or transthyretin (29) without modification of free T_4 concentrations. In pregnant ewes, only total T_4 concentrations were affected by the

treatment. This effect could be explained by a displacement of T_4 from its specific binding proteins. BPA has almost no binding property toward human TBG and a very weak one for TTR with very high dissociation constants within the micromolar range and/or very low relative potencies (30–32). Given the fact that sheep TH-binding proteins are very similar to human ones, it seems unlikely that an interaction between BPA and TH-binding proteins might explain our results.

In vitro studies indicate that BPA might alter thyroid homeostasis by modifying TH signaling pathways (15, 17, 18). This antagonistic effect of BPA on TH action was confirmed *in vivo* in amphibian models in which BPA has consistently been shown to disrupt thyroid-dependent metamorphosis (11, 12, 33) and the thyroid-induced expression of fluorescent protein in transfected *Xenopus* tadpoles or zebrafish larvae (10, 16). It is very difficult to understand how such an inhibitory effect, very likely to be associated with a certain degree of resistance to TH, could be associated with a decrease in circulating TH. Indeed, it can be assumed that this resistance should result in a decrease in feedback mechanisms leading to increased TSH and TH secretion. Thus, it seems unlikely that the decreased TH concentrations in mothers and newborn lambs after BPA treatment during pregnancy might result from a direct antagonistic action of BPA on TH signaling pathways.

A final hypothesis could rely on a possible interaction between BPA and the neuroregulation of thyroid function, resulting in an inhibition of the TRH/TSH system. Such a hypothesis would be consistent with our results in lambs. Indeed, a decreased TSH secretion would lead to a decreased synthesis and secretion of TH and more particularly T_4 , resulting in a decreased entrance rate of T_4 in blood. Such a TSH-dependent suppression of T_4 secretion should result in a simultaneous decrease in total and free T_4 concentrations in blood similar to what was observed in the newborn lambs. One study on amphibian pituitary cells indicated that BPA can potentially alter TRH-induced TSH secretion (14). However, this effect was observed only with a high concentration of BPA in the culture medium (10^{-4} M, *i.e.* 23 μ g/ml).

Whatever the mechanism of BPA-induced thyroid disruption is, it can be expected that this modification of the thyroid homeostasis will be of little consequence to the mother's health because the concentration of the free bioactive form of the hormone is maintained. The situation is a bit different for the lambs. Indeed, in the newborn, both total and free concentrations of T_4 and, to a lesser extent total T_3 , were decreased in BPA-exposed lambs. Given the critical role of TH in brain development and the exquisite sensitivity of this organ to TH, it can reasonably be assumed that we are facing a transitory alteration of thyroid

function that might have structural consequences on brain development. Accordingly, several studies in animal models report a link between developmental exposure to BPA and the occurrence of cognitive and/or behavioral alterations and/or an alteration in neural development (34–36). The critical role of THs and more particularly maternal T₄ on the development of the central nervous system is one reason for why our present results should be given full attention and warrant future studies looking at the effect BPA on thyroid function.

If we take into account the sc bioavailability, which is about 30-fold higher than the oral bioavailabilities reported in different species (37–40), it can be estimated that our dosing regimen was equivalent to an external exposure by oral route about 3000-fold higher than the current tolerable daily intake [50 µg/(kg · d) *per os*], which is itself about 10-fold higher than the highest estimated human external exposure [at maximum 4.5 µg/(kg · d) at the 95th percentile] (41). Although those observations might challenge the relevance of our results in terms of human exposure, it should be emphasized that the discrepancy between our exposure scheme and the human exposure is not that deep if we look directly at the blood concentrations rather than at the external doses. Indeed, the maximum concentrations of unconjugated BPA observed in the ewes was about 15-fold higher than the highest reported blood concentrations for unconjugated BPA in pregnant women (42). However, because of the apparent discrepancy between measured blood concentrations and known PK parameters for BPA, interpretation of the data from the standpoint of blood concentrations should be made with caution.

Independently of all the considerations regarding exposure, our study is the first to show that BPA can alter the thyroid function of pregnant animals and their offspring in a long-gestation species with similar regulation and ontogenesis of thyroid function as humans. In this context, our study is proof of concept that BPA, at least for relatively high concentrations, has a certain potential to disrupt thyroid function in fetuses and newborn animals. Even though our study cannot be considered as fully conclusive, in particular when referring to the exposure scheme that was used, it lays the foundation warranting further investigations in terms of mechanism of action of BPA on the thyroid function, mechanisms and characterization of fetal exposure throughout pregnancy, and possible consequences on central nervous system development. All those issues are key questions when addressing the potential health impact of environmental thyroid disruptors. It seems thus essential to determine whether this thyroid disruption might occur for an exposure scheme comparable to the most likely human exposure.

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